XX. *On the Persistence of Bacilli in the Gut of an Insect during Metamorphosis.* By A. Bacot, F.E.S.

[Read March 1st, 1911.]

In the course of some research work I am conducting, the possibility of a Bacillary infection of the larval gut of an insect persisting through the period of metamorphosis and continuing in the adult after emergence from the pupae is of considerable importance. I therefore infected the food of some newly hatched larvae of *Musca domestica* with a culture of *Bacillus pyocyaneus*. Puparia from this brood were sterilised outwardly by placing them in 5 per cent. to 10 per cent. solutions of lysol for five or ten minutes, then washing them in sterilised distilled water and transferring to tubes of sterile broth. They were allowed to remain in the tubes of broth for varying periods, and were then removed to a second tube of sterile broth and torn open with sterile needles. The first tube formed the control, the second the culture tube. Other puparia were passed five or six times through the flame of a Bunsen burner, and then treated in the same manner as the first batch. Growths of *pyocyaneus* were obtained in all the culture tubes and in some instances from the controls as well; these latter being cases in which the puparia had been allowed to remain in the control tubes for periods of eight or twelve hours. Adult flies that had been reared from infected puparia which had been sterilised as before mentioned, were again sterilised after their emergence, and when experimented with also gave positive results A specimen that emerged from its puparia whilst under observation was used after sterilisation with the same result, only that it gave much quicker and stronger growth, presumably because it had not been able to void the contents of the gut, which is the usual habit shortly after emergence.

These results, which have since been confirmed by Dr. Ledingham of the Lister Institute, seem to prove

conclusively that certain species of Bacilli ingested during the larval period of *M. domestica* can retain their existence whilst their host is undergoing the process of metamorphosis and continue their existence in the gut of the adult fly after emergence.

M. domestica, therefore, from its first emergence from its puparia, may be an agent in the spread of infecting organisms. It is probable that *M. domestica* is not an isolated species in this respect. The process of histolysis as described by Lowndes in regard to the Blow-fly suggests that there is no necessary bar to the continued existence of bacilli in the insect's gut from the larval to adult stages, and I have already some evidence that this may prove to be the case with insects of another order. The full details of the experiments are being published in the *Journal of Hygiene*.

There remains, however, a point, possibly of more interest to entomological than to medical science, as to the source of the infection of certain of the control tubes, after the inwardly infected but presumably outwardly sterile puparia had been allowed to soak for lengthy periods. From five to twenty-five minutes was not sufficient to cause infection, but periods of several hours or the passage through several different media seemed certain to produce infection. My own view is that this may be due to the slow passage of fluid through the stigmata of the puparia owing to an inward suction. It is noticeable that the growth arising from soakage as contrasted with pierced or cracked puparia is slow and feeble. Where puparia were passed through the Bunsen flame the growth in control was in comparison strong and rapid; this may have been due to a quicker and stronger suction through the stigmata owing to the cooling of the heated puparia in liquid or possibly to rupturing due to heat. There is another possible way by which infection might come about. When the larva shrinks into a blunt-ended oval at the close of its active existence, the mouth parts are retracted into a small pocket on the outward surface of the case; it seems possible that the sterilising fluids do not penetrate freely into this intricate passage, with the result that some organisms survive, and come in contact with the broth of the control tube if allowed to soak for any length of time. In order to prevent any possibility of infection by way of the stigmata, a further series of experiments were carried out

in which the ends of the puparia were varnished or waxed. The larvae from which the puparia were reared were again supplied with food infected with *B. pyocyaneus*, but the possibility of infection by other species of Bacteria by way of their food was not specially guarded against.

The ends of a number of puparia were varnished, and a few had the ends dipped in hot beeswax, the object being to seal the stigmata and any possible opening that might exist by way of the scar of the larval anus.

These puparia were then soaked in 10 per cent. solution of lysol or formaline for periods of from 9 to 39 hours, in most cases 10 or 12 hours. In some instances they were washed before the transference to tubes of broths, in others the washing was omitted. Events prove that washing was devoid of significance so far as the result is concerned.

After allowing the puparia to remain in the tubes of broth that formed the controls for periods of from $2\frac{1}{2}$ to $19\frac{1}{2}$ hours they were pierced or cracked in the culture tubes. In two instances second controls were used. These had respectively 14 hours first control, 12 hours second ; and 3 hours first control, $8\frac{1}{2}$ hours second.

In all, ten experiments were made : nine with varnished, and one with waxed puparia.

Every culture tube produced a growth, even after so long a sterilisation as 39 hours in 10 per cent. formaline.

Nine cases in which broth was used show clear evidence of *B. pyocyaneus* being present. One experiment in which an agar slop was used in place of the broth is not definite.

Of the controls, seven tubes were sterile, and five were infected. This number includes the two second controls, and that of the experiment where the puparia were allowed to remain in the control tube for $19\frac{1}{2}$ hours.

One tube alone, however, shows *B. pyocyaneus* in a control. In the other four instances the growth is apparently that of a strictly aerobic organism, as the broth, after the formation of a scum, became clear and gave sterile slides under the microscope. It would seem, therefore, that varnishing or waxing, if efficiently carried out, prevents the broth being infected from the interior by way of the stigmata ; and supports the contention that the infection of controls in various experiments was due to soakage through the air passages of the puparia.

The infection of the controls in these latter experiments is, I suggest, due to organisms that either resist the sterilising agent, or are protected from it for a period by the varnish or wax, and are then released by a shrinkage or partial peeling of the varnish, or wax, covering.

October 21, 1911.